IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING NATIONAL PHASE OF PCT APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

To: Hon. Commissioner of Patents
Washington, D.C. 20231

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

Atty Dkt: P 279287 /Z 70421/UST
M# /Client Ref.

From: Pillsbury Winthrop LLP, IP Group:

Date: May 7, 2001

This is a **REQUEST** for **FILING** a PCT/USA National Phase Application based on:

1. International Application	2. International Filing Date	3. Earliest Priority Date Claimed
PCT/GB99/03648	4 November 1999	10 November 1998
⇧ country code	Day MONTH Year	Day MONTH Year (use item 2 if no earlier priority)

4. Measured from the earliest priority date in item 3, this PCT/USA National Phase Application Request is being filed within:

(a) ☐ 20 months from above item 3 date (b) ☒ 30 months from above item 3 date,

(c) Therefore, the due date (unextendable) is May 10, 2001

5. Title of Invention METHODS FOR IDENTIFYING MODULATORS OF BS69 ACTIVITY

6. Inventor(s) GREEN, Isabelle et al

Applicant herewith submits the following under 35 U.S.C. 371 to effect filing:

7. ☒ Please immediately start national examination procedures (35 U.S.C. 371 (f)).

8. ☒ **A copy of the International Application** as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (file if in English but, if in foreign language, file only if not transmitted to PTO by the International Bureau) including:

a. ☒ Request;
b. ☒ Abstract;
c. 21 pgs. Spec. and Claims;
d. _____ sheet(s) Drawing which are ☐ informal ☐ formal of size ☐ A4 ☐ 11"

9. ☒ **A copy of the International Application has been transmitted by the International Bureau.**

10. **A translation of the International Application** into English (35 U.S.C. 371(c)(2))

a. ☐ is transmitted herewith including: (1) ☐ Request; (2) ☐ Abstract;
(3) _____ pgs. Spec. and Claims;
(4) _____ sheet(s) Drawing which are:
☐ informal ☐ formal of size ☐ A4 ☐ 11"

b. ☐ is not required, as the application was filed in English.

c. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

d. ☐ Translation verification attached (not required now).

JC08 Rec'd PCT/PTO 07 MAY 2001

11. ☒ Please see the attached Preliminary Amendment

12. ☐ Amendments to the claims of the International Application **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., before 18th month from first priority date above in item 3, are transmitted herewith (file only if in English) including:**

13. ☒ PCT Article 19 claim amendments (if any) have been transmitted by the International Bureau

14. ☐ Translation of the amendments to the claims **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., of claim amendments** made before 18th month, **is attached (required by 20th month from the date in item 3 if box 4(a) above is X'd, or 30th month if box 4(b) is X'd, or else amendments will be considered canceled).**

15. **A declaration of the inventor** (35 U.S.C. 371(c)(4))
a. ☐ is submitted herewith ☐ Original ☐ Facsimile/Copy
b. ☒ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

16. **An International Search Report (ISR):**
a. Was prepared by ☐ European Patent Office ☐ Japanese Patent Office ☐ Other
b. ☒ has been transmitted by the international Bureau to PTO.
c. ☒ copy herewith (3 pg(s).) ☒ plus Annex of family members (3 pg(s).).

17. **International Preliminary Examination Report** (IPER):
a. ☒ has been transmitted (if this letter is filed after 28 months from date in item 3) in English by the International Bureau with Annexes (if any) in original language.
b. ☒ copy herewith in English.
c.1 ☐ IPER Annex(es) in original language ("Annexes" are amendments made to claims/spec/drawings during Examination) including attached amended:
c.2 ☐ Specification/claim pages #_____ claims #
Dwg Sheets #
d. ☐ Translation of Annex(es) to IPER **(required by 30th month due date, or else annexed amendments will be considered canceled).**

18. **Information Disclosure Statement** including:
a. ☒ Attached Form PTO-1449 listing documents
b. ☐ Attached copies of documents listed on Form PTO-1449
c. ☒ A concise explanation of relevance of ISR references is given in the ISR.

19. ☐ **Assignment** document and Cover Sheet for recording are attached. Please mail the recorded assignment document back to the person whose signature, name and address appear at the end of this letter.

20. ☐ Copy of Power to IA agent.

21. ☐ **Drawings** (complete only if 8d or 10a(4) not completed): ___ sheet(s) per set: ☐ 1 set informal; ☐ Formal of size ☐ A4 ☐ 11"

22. Small Entity Status ☒ is **Not** claimed ☐ is claimed (**pre**-filing confirmation required)
22(a) _____ (No.) Small Entity Statement(s) enclosed (since 9/8/00 Small Entity Statements(s) not essential to make claim)

23. **Priority** is hereby claimed under 35 U.S.C. 119/365 based on the priority claim and the certified copy, both filed in the International Application during the international stage based on the filing in (country) GREAT BRITAIN of:

	Application No.	Filing Date		Application No.	Filing Date
(1)	9824501.2	November 10,1998	(2)		
(3)			(4)		
(5)			(6)		

a. ☒ See Form PCT/IB/304 sent to US/DO with copy of priority documents. If copy has not been received, please proceed promptly to obtain same from the IB.
b. ☐ Copy of Form PCT/IB/304 attached.

RE: USA National Phase Filing of PCT/GB99/03648 JC08 Rec'd PCT/PTO 07 MAY 2001

24. Attached: 9 pages of Sequence Listing and 2 copies of Form PCT/IB/306

25 Per Item 17.c2, **cancel original** pages #_____, claims #_____, Drawing Sheets #

26. **Calculation of the U.S. National Fee (35 U.S.C. 371 (c)(1)) and other fees is as follows:**
Based on amended claim(s) per above item(s) ☐ 12, ☐ 14, ☐ 17, ☐ 25 (hilite)

Total Effective Claims	minus 20 =	x $18/$9	=	$0	966/967
Independent Claims	minus 3 =	x $80/$40	=	$0	964/965
If any proper (ignore improper) Multiple Dependent claim is present,		add$270/$135		+0	968/969

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): →→ **BASIC FEE REQUIRED, NOW** →→→→

A. If country code letters in item 1 are **not** "US","BR","BB","TT","MX","IL" "NZ", "IN" or "ZA"

See item 16 re:

1.	Search Report was not prepared by EPO or JPO - - - - - - - - -	add$1000/$500		960/961
2.	Search Report was prepared by EPO or JPO - - - - - - - - - - -	add$860/$430	+860	970/971

SKIP B, C, D AND E UNLESS country code letters in item 1 are "US","BR","BB","TT","MX","IL", "NZ", "IN" or "ZA"

☐(X)	B. If USPTO did not issue both International Search Report (ISR) and (if box 4(b) above is X'd) the International Examination Report (IPER), - - - - - - - - - - - - - - - - - - -	add$1000/$500	+0	960/961
☐(only) ☐(one) →	C. If USPTO issued ISR but not IPER (or box 4(a) above is X'd), -	add$710/$355	+0	958/959
☐(of) ☐(these) ☐(4) → ☐(boxes)	D. If USPTO issued IPER but IPER Sec. V boxes not all 3 YES, -	add$690/$345	+0	956/957
→ ☐	E. If international preliminary examination fee was paid to USPTO and Rules 492(a)(4) and 496(b) satisfied (IPER Sec. V all 3 boxes YES for all claims), - - - - - - - - - - - - -	add $100/$50	+0	962/963
27.		**SUBTOTAL =**	$860	
28.	If Assignment box 19 above is X'd, add Assignment Recording fee of ----$40		+0	(581)
29.	Attached is a check to cover the -	**TOTAL FEES**	$860	

Our Deposit Account No. 03-3975
Our Order No. 9901 (C#) | 279287 (M#)

00909

CHARGE STATEMENT: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 and 492 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Account/Order Nos. shown above for which purpose a duplicate copy of this sheet is attached.
This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal form is filed

Pillsbury Winthrop LLP
Intellectual Property Group

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Atty/Sec: DJB/mhn

NOTE: File in duplicate with 2 postcard receipts (PAT-103) & attachments.

09/831143
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

Inventor(s): GREEN, Isabelle et al

Filed: Herewith

Title: METHODS FOR IDENTIFYING MODULATORS OF BS69 ACTIVITY

May 7, 2001

PRELIMINARY AMENDMENT

Hon. Commissioner of Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

IN THE SPECIFICATION:

At the top of the first page, just under the title, insert

☒ --This application is the National Phase of International Application PCT/GB99/03648 filed November 4, 1999 which designated the U.S. and that International Application ☒ was ☐ was not published under PCT Article 21(2) in English.--

Respectfully submitted,

PILLSBURY WINTHROP LLP
Intellectual Property Group

By: _____

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APPLICATION UNDER UNITED STATES PATENT LAWS

Atty. Dkt. No. PW 279287/Z70421/UST
(M#)

Invention: METHODS FOR IDENTIFYING MODULATORS OF BS69 ACTIVITY

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This is a:

☐ Provisional Application

☐ Regular Utility Application

☐ Continuing Application
☒ The contents of the parent are incorporated by reference

☒ PCT National Phase Application

☐ Design Application

☐ Reissue Application

☐ Plant Application

☐ Substitute Specification
Sub. Spec Filed ____________
in App. No. ______/______

☐ Marked up Specification re
Sub. Spec. filed ____________
In App. No ______/______

SPECIFICATION

METHODS FOR IDENTIFYING MODULATORS OF BS69 ACTIVITY

A new modulator of the Transforming Growth Factor-β (TGF-β) cell signalling pathway is described, namely an endogenous human protein designated BS69. Methods are provided to identify compounds that interfere with the biological activity of BS69 on the TGF-β cell signalling pathway.

TGF-β itself regulates, *inter alia*, cell proliferation and differentiation, gene expression, embryonic development, extracellular matrix formation, haematopoiesis, apoptosis, wound healing, bone development and immune and inflammatory responses. The multiple effects of TGF-β lead to the medical need for both agonists and antagonists of its action. Jackson (Exp. Opin. Ther. Patents. (1998) **8(11)**:1479-1486) reviews key patents and scientific publications directed to modulation of the activity of TGF-β.

TGF-β cell signalling failure is implicated in a number of different tumour types and in the generation of human fibrotic disorders. Cell signal failure within other receptors in the TGF-β super family is implicated in other diseases such as arthritis, atherosclerosis, apoptosis, inflammation, wound healing and diabetic nephropathy. It is also known that administration of TGF-β helps prevent mucosistis and alopecia in patients undergoing chemotherapy or radiotherapy (PCT Publication No. 94/06459) and lowers resitance of multi-drug resitant malignant cells to chemotherapy (PCT Publication No. 92/13551).

TGF-β is one growth factor of a large super family which play broad roles in cell growth and differentiation in a variety of organisms. Examples of groups within the super family are the TGF-β set, activin/inhibin set, Mullerian inhibiting substance, glial cell line-derived neurotrophic factor and Bone Morphogenetic Proteins (BMPs).

Within the TGF-β subset the biological response to the growth factor is first initiated by binding of TGF-β to its respective receptor. The receptor is formed from two components TGF-β receptor I (TβR-I) and TGF-β receptor II (TβR-II) both of which contain cytoplasmic serine/threonine kinases and both of which are required for effective cell signalling (Wieser, R. et al., Mol. Cell. Biol. (1993) **13**:7239-7247).

It was not until recently that the biological molecules involved in signal cell transduction of TGF-β were discovered. Through screening of genes in transgenic Drosophila expressing only partially active decapentaplegic (DPP), which is equivalent to BMP in

vertebrates, a new gene was found [Mothers against DPP(Mad)] which was able to restore the phenotype to the transgenic Drosophila expressing only partially active DPP protein (Sekelsky et al. Genetics (1995) **139**:1347-1359). Analysis of the Mad gene sequence showed it to be closely homologous to the sma genes of *Caenorhabditis elegans* and the putative human tumour suppressor gene DPC4 (Deleted in Pancreatic Carcinoma). Such genes, of which several have now been identified, are now collectively referred to as Smad (Derynck, R. et al., Cell (1996) **87**:173).

The Smad proteins constitute a unique signalling pathway which convey signals directly from TGF-β type receptors to the nucleus, where they modulate gene transcription. There is close homology between many of the Smad proteins across species to such a degree that Smad proteins from one species may elicit a response in a different species. Many Smad proteins (Smad 1, 2, 3, 5 and others) are specific to the pathway associated with a particular receptor, others (Smad 4) act as a common mediator in different pathways, others (Smad 6 and 7) are inhibitory Smads that bind TGF-β receptor and block phosphorylation of the specific Smads. Current understanding of the TGF-β signalling pathway is briefly described below, a full review can be found in Heldin et al., (Nature (1997) **390**: 465).

In brief, the mechanism of receptor cell signal transduction involves the following steps. For TGF-β signalling, the TβRI and II receptors are activated by autophosphorylation following TGF-β binding to the receptor complex. Smad 2 or 3 associate with the activated receptor complex and are themselves phosphoylated at a characteristic C-terminal Ser-Ser-X-Ser motif. After activation, the Smad 2/3 forms a stable complex with the common mediator Smad 4, which in turn translocates to the nucleus where it directly or indirectly modulates gene transcription.

As described above the TGF-β signalling pathway is implicated in a number of different diseases and as such this biological mechanism represents an attractive target for intervention in treating such diseases.

BS69 (Hateboer, R. et al., EMBO Journal (1995) **14(13)**:3159-3169 and PCT Publication No. WO 97/00323) is described as being an adenovirus EI-A-associated protein which inhibits EIA adenovirus gene transactivation. A later disclosure (Kurozumi, K. et al., (1998) **3(4)**:257-264) describes an alternatively spliced, and considerably shorter, form of

BS69, which they call BRAM1 (BMP Receptor Associated Molecule 1), as being able to complex intracellularly with the BMP receptor.

We have found through the use of protein hybridisation studies that the protein BS69 complexes with the Smad 2 and 3 proteins.

Whilst BMP is a member of the TGF-β superfamily of growth factors/receptor types it is known that BMP does not elicit signal transduction through Smad 2 or 3 proteins. In addition Kurozumi et al. found that full length BS69 did not complex with the BMP receptor and, therefore, no cellular function for the BS69 protein was described. Indeed, Kurozumi et al. remark "Therefore, the function of BRAM-1 may be different from that of BS69. At present, the cellular function of BS69 is not known". The present inventors have discovered a new cellular mechanism of action for the BS69 protein.

We present as the first feature of the invention a method for the discovery of a modulator of BS69 activity, which method comprises contacting an assay system capable of presenting information on the effects of a chemotherapeutic agent on the activity of BS69 or a derivative thereof with a potential chemotherapeutic agent under conditions in which BS69 is active in the absence of the potential chemotherapeutic agent and measuring the extent to which the potential chemotherapeutic agent is able to modulate the activity of BS69.

There is therefore provided, a method for identifying modulators of BS69 activity, which method comprises contacting an assay system, capable of presenting information on the effects of a test compound on the activity of BS69 or a derivative thereof, with a test compound and measuring the activity of BS69.

Preferably BS69 activity may be described as the binding of BS69, or a fragment thereof to a human BS69 binding substrate. A "human BS69 binding substrate" is a protein endogenously expressed in human cells which is capable of having its biological function modulated by binding of BS69. For the avoidance of doubt, adenovirus E1A protein is not a human BS69 binding substrate. Preferably the human BS69 binding substrate is selected from Smad 2, Smad 3, a complex of Smad 2 and Smad 4, and a complex of Smad 3 and Smad 4, or individual fragments thereof (herein after called BS69 binding substrate), more preferably the BS69 binding substrate is Smad 2 or Smad 3, or fragments thereof capable of binding BS69. A "human BS69 binding substrate" may also be a nucleic acid to which BS69 or a protein complex comprising BS69 binds, such as a BS69 transcription factor dependent

promoter or other regulatory elements which are affected directly or indirectly by binding of BS69. A preferred promoter element whose regulation is controlled in part by BS69 is plasminogen activator inhibitor-1 (PAI-1).

Thus according to a further aspect of the invention there is provided a method for the discovery of a modulator of BS69 activity, which method comprises contacting an assay system capable of presenting information on the effects of a chemotherapeutic agent on the binding of BS69 or a derivative thereof to a human BS69 binding substrate with a potential chemotherapeutic agent under conditions in which BS69 binds to the human BS69 binding substrate in the absence of the potential chemotherapeutic agent and measuring the extent to which the potential chemotherapeutic agent is able to modulate the activity of BS69.

According to a further aspect of the invention there is provided a method for the discovery of a modulator of BS69 activity, which method comprises contacting an assay system capable of presenting information on the effects of a chemotherapeutic agent on the binding of BS69 or a derivative thereof to a BS69 binding protein with a potential chemotherapeutic agent under conditions in which BS69 binds to the BS69 binding protein in the absence of the potential chemotherapeutic agent and measuring the extent to which the potential chemotherapeutic agent is able to modulate the activity of BS69.

According to a further aspect of the invention there is provided a method of screening for an agent useful in treating disorders characterised by an abnormality in a TGF-β signalling pathway, wherein said pathway involves an interaction between BS69 and a human BS69 binding partner, comprising screening potential agents for ability to disrupt or promote said interaction as an indication of a useful agent.

Potential chemotherapeutic agents which may be tested in the screen include those molecules, whether simple organic molecules, for example, of less than 2000 Daltons or larger biologic molecules, such as peptides, antibodies or DNA/RNA sequences, which may modulate the biology or pharmacology of BS69 activity, for instance by affecting the protein:protein binding of BS69 to a human BS69 binding substrate or by modulating the expression of DNA or RNA which encodes BS69. Suitable molecules include simple organic molecules, mimetics, nucleotide sequences, antibodies and any other molecules that modulate the activity of BS69. Chemotherapeutic agents/test compounds include both chemical and biological molecules.

It will be appreciated that there are many assay systems which may be employed to perform the present invention. Examples of assay systems used to detect agents which may modulate the biological or pharmacological activity of BS69 are:

In vitro proximity assays such as a scintillation proximity assay (SPA), as described in Udenfield et al., (Anal. Biochem. (1987) **161**:494). In SPA derivatised microspheres which contain a scintillant and a fluorophore are used which attach through the derivatised group to a biological molecule of interest. When the biological molecule of interest binds a radiolabelled molecule then the proximity of the radiolabel to the scintillant causes increased emission of radiation signal and measurable increases in fluorophore excitation. In the present case, for example, BS69 is bound to a scintillant/fluorophore containing microsphere through, for example, a streptavidin/biotin bridge, and a human BS69 binding substrate is radiolabelled or bound to a support with a radiolabel. Any potential chemotherapeutic agent which affects the way in which BS69 binds to the human BS69 binding substrate will affect the radiation emitted by the system. Alternatively instead of radiolabels and scintillants, fluorophore donor and acceptor molecules may be used in what is called homogeneous time resolved fluorescence (HTRF), for example the acceptor fluorophore can be XL 665 and the donor fluorophore is europium (CIS Bio.). A further preferred feature of the invention is the invention as defined above wherein the assay system is a proximity assay, preferably SPA or HTRF.

In vitro cellular assay systems may be used. For example, a measurable output of BS69 activity could be detected when a reporter gene is placed under the control of the TGF-β signal transduction pathway. A stable cell is created which has a reporter gene under transcriptional control of the TGF-β pathway and which also expresses BS69, such a cellular assay may be prepared as described in US 5,436,128. Genes under TGF-β control which may be replaced by a reporter gene, for example by homozygous recombination, include plasminogen activator inhibitor-1, $p15^{mk4b}$, and p^{WAF1}, (Attisano et al., Biochemica et Biophysica Acta (1994) **1222**:71-80; Hannon and Beach, Nature (1994) **371**:257-261; and, Datto et al., J.Biol.Chem., (1995) **270**:28623-28628). In this way, stimulation or inhibition of signal transduction results in stimulation or inhibition of reporter gene activity and potential test agents which interfere with BS69 activity may be detected. Suitable reporter genes include the β-galactosidase lac Z gene of *E. coli* (Casadaban et al., Meth. Enzymol. (1983)

100:293-308) or the firefly luciferase gene (de Wet et al., Proc. Nat. Acad. Sci. USA (1985) 82:7870-7873).

We present as a feature of the invention a method for the discovery of a modulator of BS69 activity, which method comprises contacting a potential chemotherapeutic agent with a cell comprising a reporter gene, expression of the reporter gene being under the control of the TGF-β signal pathway which is in turn under the control of BS69, a promoter which is activated by the TGF-β signal pathway and which has the gene encoding the reporter protein under its control, and determining modulation of BS69 by the potential chemotherapeutic agent by reference to any change in the expression of the reporter gene. Preferably the measurement of reporter gene expression is compared with a control cell construct wherein the reporter gene is under the control of the TGF-β signal pathway but in which BS69 is not expressed. The cell is preferably a mammalian cell, more preferably a stably transfected cell or cell line.

The promoter may be a naturally occurring promoter for TGF-β signalling, or it may be a synthetic promoter responsive to the TGF-β transduction pathway. Synthetic promoters would comprise one or more response elements to the signalling pathway, as well as elements such as a TATA box, required for correct transcription initiation. A preferred promoter is plasminogen activator inhibitor-1 (PAI-1).

The components of the TGF-β signalling pathway may be endogenously expressed within the cells used in such assay, for example by the use of mammalian cell lines. Alternatively, components, such as heterologous receptors, may be expressed so that they couple to the TGF-β signalling pathway. Also, an endogenous component may be removed, for example by gene deletion, and replaced with an exogenous protein which will restore the function of the pathway.

An alternative *in vitro* cellular system is the two-hybrid assay system. The two-hybrid system uses the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA-binding site that regulates the expression of a reporter gene. Commercially available systems such as the CLONTECH, Matchmaker™ systems and protocols may be used with the present invention. (*See also*, Mendelsohn, A.R., Brent, R., Curr. Op. Biotech., **5**:482 (1994); Phizicky. E.M. and Fields, S., Microbiological Rev., **59(1)**:94 (1995); Yang, M., et al., Nucleic Acids Res., **23(7)**:1152 (1995); Fields, S. and

Sternglanz, R., TIG, **10(8)**:286 (1994); US Patents 5,283,173 and 5,468,614). Two hybrid screening systems can be practised either with a positive readout or with a negative readout. Recently, some examples of "reverse" two-hybrid systems have been described. Leanna, C.A. and Hannick, M. (Nucl. Acids Res. (1996) **17**:3341-3347) use an output in which a gene under the control of the two hybrid system is toxic in the presence of cycloheximide. Vidal, M., Brachmann, R., K., Fattaey, A., Harlow, E. and Boeke, J.D. (Proc. Natl Acad. Sci. U.S.A. (1996) **93**:10315-10320) use the property of the URA3 gene product that it can be selected against by 5-fluoro-orotic acid. It is possible to test the ability of a potential chemotherapeutic agent to interfere with the binding of BS69 and a BS69 binding substrate, where BS69 is expressed as a fusion protein to a part of a transcription factor, either the transcription activation domain or the DNA-binding site, and the human BS69 binding substrate is expressed as a fusion protein to the other part of the transcription factor. Such that, if hybridisation of the transcription factor is prevented from occurring by a chemotherapeutic agent then transcription of a reporter gene under transcriptional control of the transcription factor is interrupted.

Several variations on the two hybrid system are known, and may be configured for use in the present invention. For example, a "tribrid" system has been described in which the two hybrid interaction will only occur if one component is phosphorylated by a kinase introduced into the cell (Osborne, M.A., Dalton, S. and Kochan, J.P. (1995) Bio/Technology 13, 1474-1478).

The two hybrid or tribrid systems can be adapted for use in yeast or, preferably, mammalian cells.

We present as a feature of the invention a method for the discovery of a modulator of BS69 activity, which method comprises contacting a potential chemotherapeutic agent with a cell comprising a transcription factor dependant promoter, a reporter gene under the control of the transcription factor dependant promoter, a fusion protein of BS69, or a human BS69 binding substrate, and a domain of a transcription factor which binds to the promoter and a second fusion protein of a human BS69 binding substrate, or BS69, and a domain of the transcription factor which activates transcription, wherein binding of BS69 to the human BS69 substrate causes the two domains of the transcription factor to become disposed to promote expression of the reporter gene, and determining modulation of BS69 activity by the

potential chemotherapeutic agent by reference to any change in the expression of the reporter gene. It will be apparent that in the above system one of the fusion proteins will have a BS69 binding component and the other will have a BS69 binding substrate component.

As described above, an alternative approach to the intervention of BS69 binding to a BS69 binding substrate is to affect gene transcription or gene translation in the cell and thus prevent BS69 protein production in the cell. A variety of points in these processes may be disrupted such as by interference by a chemotherapeutic agent in the binding of BS69 transcription factors to the upstream promoter sites or by a chemotherapeutic agent binding to the coding DNA or mRNA (such as anti-sense nucleotides) of BS69.

Assay methods which may be utilised in the performance of the above aspect of the invention include those disclosed in European Publication No. 0483249.

Compounds that modulate the expression of DNA or RNA encoding the BS69 polypeptide may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression of a reporter gene. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample. Systems in which transcription factors are used to stimulate a positive output, such as transcription of a reporter gene, are generally referred to as "one-hybrid systems" (Wang, M.M. and Reed, R.R. (1993) Nature **364**:121-126). Using a transcription factor to stimulate a negative output (growth inhibition) may thus be referred to as a "reverse one-hybrid system" (Vidal et al, 1996, *supra*). Therefore, in an embodiment of the present invention, a reporter gene is placed under the control of a BS69 promoter.

In a further aspect of the invention we provide a heterologous cell wherein expression in the cell of a reporter gene is under the control of a BS69 transcription factor dependent promoter, and wherein expression of the transcription factor is inducible, whereby inhibition of gene transcription by the potential chemotherapeutic agent may be determined by reference to a lack of expression of the reporter gene.

In a further aspect of the invention we provide a cell or cell line comprising a reporter gene under the control of a BS69 transcription factor dependent promoter.

We also provide a method for identifying inhibitors of BS69 transcription which method comprises contacting a potential therapeutic agent with a cell or cell line as described

above and determining inhibition of BS69 transcription by the potential therapeutic agent by reference to a lack or reduced expression of the reporter gene.

A method for identifying modulators (activators or inhibitors) of BS69 transcription which method comprises contacting a cell or cell line as described above, said cell or cell line also supplied with exogenous or endogenous BS69, with a test compound and determining the effect on BS69 transcription by the test compound by reference to enhanced or reduced expression of the reporter gene.

In general, eukaryotic transcription factors consist of a DNA binding domain and a transcription activation domain (Ptashne (1988) Nature **335**:683-689). Frequently these factors are dimers. Thus there may be three interfaces at which interference with a chemotherapeutic agent may inhibit a transcription factor: the DNA:protein interface, the dimerisation interface, and the interface between the activation domain and the transcription apparatus (Peterson, M.G. and Baichwal, V.J. (1993) TibTech **11**:11-18). To find inhibitors of the interaction of mammalian DNA binding protein with its binding site, a transcription activation domain is fused to said DNA binding domain in order to make a transcription factor which functions in the cell type of interest. Conversely, if the interaction between an activation domain and the transcription machinery is of interest, a DNA binding domain may be fused to the activation domain of interest to yield a transcription factor. In such circumstances, it may also be desirable to express within the cell the protein which the activation domain contacts. Generally, activation domains are believed to activate transcription through recruitment of the RNA polymerase holoenzyme (Ptashne, M. and Gann, A. (1997) Nature **386**:569-577). This recruitment occurs through protein:protein interactions. Using genetic techniques it is possible to substitute components of the *S. cerevisiae* holoenzyme for mammalian homologues. In this way the protein:protein interaction of interest may be reconstituted using components from the same species.

Reference to BS69 its polypeptide, DNA or RNA sequences include references to derivatives thereof. Derivative polypeptides, or DNA/RNA sequences, of BS69 include:

i) allelic variations of BS69, in particular any single nucleotide polymorphism (SNP);

ii) a fragment of BS69, i) or iii) capable of binding to BS69 binding substrate; and

iii) a mutant form of BS69, i) or ii),

and, preferably, exclude BRAM1.

Particularly preferred polypeptide fragments are those which are at least 15 amino acid long and include wholly or at least partially the Smad binding domain of BS69. The Smad binding domain has been localised to between about amino acid 450 and the C-terminus of the BS69 protein (aa 562) disclosed as SEQ ID No. 2 in PCT Publication No. WO 97/00323. Thus, preferred fragments or polypeptides of BS69 include this region. In WO 97/00323 the E1A binding domain was localised to between amino acids 412 and 532. We have found that the Smad2 binding domain is contained within amino acids 443-562. It is evident that the area of BS69 that mediates interaction with E1A also mediates interaction with Smad2. It is likely therefore that the domain lies within the region defined by amino acids 443 and 512. Particularly preferred fragments for use in the invention include the BS69 polypeptide sequence from amino acids 450-562 or 443-562 or 443-512 or 450-512.

For the purposes of this application the nucleic acid and amino acid sequence of BS69 referred to herein are disclosed in PCT Publication No. WO 97/00323 SEQ ID NO:1 and 2.

Allelic variations or SNPs in the BS69 DNA sequence may be detected by alteration in the pattern of restriction fragment length polymorphisms capable of hybridising to SEQ ID NO:1 of WO 97/00323 or by the inability of allele-specific oligonucleotide probes to specifically hybridise to SEQ ID NO:1 of WO 97/00323 under appropriate conditions. BS69 SNPs can also be determined by nucleic acid sequencing.

It will be readily appreciated by the skilled reader that as a result of the degeneracy of the genetic code, a multitude of sequences some having minimal homology (sequence identity) to any naturally occurring gene for BS69, may be produced and found to have utility in the present invention. Thus, the invention contemplates each and every possible variation of nucleotide sequence based on possible codon choices coding for the same amino acid.

Monospecific antibodies to BS69 may be purified from mammalian antisera containing antibodies reactive against the polypeptide or are prepared as monoclonal antibodies reactive with the BS69 using the technique of Kohler and Milstein, (Nature, (1975) **256**:495). Mono-specific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for BS69. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope. BS69 specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with rabbits being preferred, with an

appropriate concentration of BS69 either with or without an immune adjuvant and optionally conjugated to a carrier protein such as albumin.

Further features of the invention include:

A method of treatment of a patient in need of such treatment for a condition which is mediated by the biological or pharmacological activity of BS69 on a human BS69 binding substrate, comprising administration of a polypeptide substantially as depicted in WO97/00323 SEQ ID NO:2 or a pharmacologically active fragment thereof.

A method of treatment of a patient in need of such treatment for a condition which is mediated by the biological activity of BS69 on a human BS69 binding substrate, comprising administration of a nucleic acid substantially as depicted in WO97/00323 SEQ ID NO:1 or the anti-sense sequence or a biologically-effective fragment of either thereof.

A method of treatment of a patient in need of such treatment for a condition which is mediated by the biological activity of BS69 on a human BS69 binding substrate, comprising administration of an antibody against BS69 substantially as depicted in WO97/00323.

A compound that modulates the biological or pharmacological activity of BS69 on a human BS69 binding substrate identified by the method of the invention as described above.

A compound that modulates BS69 transcription or other BS69 activity, identified according to the methods of the invention as described above.

A pharmaceutical composition comprising a compound that modulates the biological or pharmacological activity of BS69 on a human BS69 binding substrate identified by the method of the invention as described above.

A method of treatment of a patient in need of such treatment for a condition which is mediated by the pharmacological or biological activity of BS69 on a human BS69 binding substrate comprising administration of a modulating compound or pharmaceutical composition thereof identified by the method of the invention as described above.

Use of a polypeptide, nucleic acid, antibody or any other therapeutic agent substantially as depicted in WO97/00323, in the manufacture of a medicament for treating diseases mediated by TGF-β, particularly abnormal TGF-β expression.

The teaching in WO 97/00323 is incorporated herein by reference.

Example 1

Identifying BS69 as a modulator of the TGF-β signalling pathway.

In order to identify novel modulators of the TGF-beta signalling pathway we employed the two hybrid screening methodology. The two hybrid system can be used in order to detect expressed proteins from a cDNA library that interact with a protein of interest. The protein that we used as bait was Smad2. Smad2 is one of the pathway specific Smads and is known to lie on the TGF-beta pathway from receptor to nucleus. Smad2 has been shown to be in a complex with Smad4 and Fast-1 on the TGF-beta inducible Xenopus promoter, Mix.2 .

Two Hybrid Screen Construction

Human full length Smad2 was isolated by PCR from a human brain cDNA library (OriGene Technologies, Rockville MD.) and cloned into PCRScript® (Stratagene). SEQ ID Nos. 1 and 2 were the oligonucleotide primers used for the PCR synthesis.

The full length human Smad2 insert was excised from the PCRScript® using Sma I and Sal I restriction endonucleases and cloned into the two hybrid bait vector pGBD-1 (James, Genetics (1996) **144**:1425-1436) resulting in a N-terminal Gal4 DNA binding domain fusion with full length human Smad2 (Gal4::Smad2). In order to verify that full length Smad2 was interaction competent, Xenopus Fast-1 and human Smad4 were cloned into the activation domain fusion vector pGAD-1 (AD::Fast1, AD::Smad4). It had been previously shown that these proteins will interact with human Smad2 (Chen, Nature (1997) **398(4)**:85-88). As such, the Smad2/Fast-1 and Smad2/Smad4 interaction was used as a positive control for the ability of Smad2 to interact with other proteins in the yeast two hybrid system.

Human full length Smad4 was isolated in a similar manner to Smad2 from a human skeletal muscle cDNA library using oligonucleotide primers corresponding to SEQ ID Nos. 3 and 4.

Xenopus Fast-1 was inserted into PCRScript® by amplification of a cloned Xenopus Fast-1 sequence (C.Hill, ICRF, London) and then excised with Sma I and Bgl II restriction

endonuclease and cloned into pGAD-1. The primers used for the PCR reaction were SEQ ID Nos. 5 and 6.

As a negative control human Smad1 was isolated and cloned into pGAD-1 (AD::Smad1). Smad1 does not interact with Smad2 *in vivo* or *in vitro* (Zhang et al. Nature (1996) **383**:168-172; Lagna, G., et al Nature (1996) **383**:832-836). The primers used for the PCR reaction were SEQ ID Nos. 7 and 8.

The yeast two hybrid system used is that of Vidal et al., (PNAS USA (1996) **93**:10321). The *S. cerevisiae* screening strain, MAV203, has three reporter genes (HIS3, URA3, and LacZ) stably integrated in single copy numbers at different loci in the yeast genome. Interaction of an activation domain fused protein with a DNA bound protein of interest will result in induction of the His3, Ura3, and LacZ reporter genes allowing growth of MAV203 on medium lacking histidine and uracil, and producing blue colonies when assayed with X-Gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside). MAV203 was transformed with combinations of the different control plasmids and were scored on their ability to grow on medium lacking uracil and histidine, and producing blue colour. This was done on a scale of +++, meaning very high, to -, meaning none.

Table 1 indicates that when GAL4::Smad2 is transformed into MAV203 on its own it cannot activate any of the reporter genes. If Smad2 is co-transformed with AD::Fast-1 a strong interaction is observed between the two as indicated by very high activation of the reporter genes. A similar strong interaction is observed between the Smad2 and AD::Smad4 fusion proteins in the two hybrid assay. Contrasting this, no interaction was observed between Smad2 and AD::Smad1 and none of the activation domain tagged proteins were able to activate the reporter genes on their own. This indicates that the GAL4::Smad2 fusion protein is functional in its ability to interact with other proteins.

A screen was then performed in order to identify proteins that interact with Smad2. MAV203 was co-transformed with GAL4::Smad2 and a human skeletal muscle two hybrid cDNA library (Clonetech). 5.9 x 10^6 independent co-transformants were assayed for their ability to interact with Smad2. Five different proteins were isolated and showed varying ability to interact with GAL4::Smad2 from very strong (+++, 1 isolate), strong (++, 2 isolates) and weak (+, 2 isolates) as assayed by their ability to activate all three reporter genes. The

cDNA containing plasmids were isolated and co-transformed with GAL4::Smad2 as a check for "true positive". All five retained that ability to activate the three reporter genes.

The strongest interactor, Cl.51, was isolated independently in the screen 33 times. Sequence analysis of the longest isolate of Cl.51 indicated that it was a previously identified protein BS69. BS69 was previously identified as a protein that interacts with the adenoviral 289R E1A gene product and in doing so inhibits its ability to activate transcription (Hateboer, R. et al., EMBO J. (1995) **14(13)**:3159-3169). BS69 is a 562 amino acid protein and has a single corresponding mRNA species of approximately 4.7 kb. The longest isolate of BS69 in the present two hybrid screen was 2200bp, encoding the C terminal region of the protein from amino acid 450 to 562 and the 3' untranslated region. This area of the C terminus of BS69 contains the E1A interaction domain (Hateboer, R. et al., EMBO J. (1995) **14(13)**:3159-3169). It is therefore evident that the area of BS69 that mediates interaction with E1A also mediates interaction with Smad2.

In order to assess the specificity of the BS69/Smad2 interaction, the other members of the Smad family, as well as a totally unrelated protein, peroxisome proliferating antigen receptor gamma (PPAR-G), were examined for protein-protein interaction with BS69 in the two hybrid system. Each of the proteins were cloned from PCR reactions in a manner similar to that for Smad2 into the pGBD-1 vector in order to make GAL4 DNA binding domain fusions. Smad3 was cloned using PCR primers corresponding to SEQ ID Nos. 9 and 10. Smad5 was cloned using PCR primers corresponding to SEQ ID Nos. 11 and 12. Smad6 was cloned using PCR primers corresponding to SEQ ID Nos. 13 and 14. Smad 7 was cloned using PCR primers corresponding to SEQ ID Nos. 15 and 16.

Table 2 summarises the results from the BS69 specificity two hybrid analysis. BS69 interacts very strongly with Smad2 and Smad3. A very weak (+/-) interaction is obtained with Smad1 which may not be physiologically relevant. No interaction is observed with Smad4, Smad5, Smad6, or Smad7. BS69 does not interact with PPAR-G and cannot activate the reporter genes on its own. This indicates that the BS69 interaction observed is specific to Smad2 and Smad3 (possibly Smad1) suggesting a role for BS69 in modulating the activity of TGF-beta through Smad2 and/or Smad3. As BS69 was identified as a protein that interacts with E1A, inhibiting its ability to activate transcription, the same may be true in relation to the TGF-beta pathway. Smad2 and Smad3 have both been shown to contain transcription

activation function. They heterodimerise with Smad4, translocate to the nucleus, and activate TGF-beta responsive genes. BS69 may function cellularly as an inhibitor of TGF-beta induced transcriptional activation by interacting with Smad2/Smad3 and inhibiting their ability to activate transcription.

It will be apparent to the person skilled in the art that use of the specific vectors and strains as described in this example is not essential. Other commercially available yeast two hybrid systems, using slightly different vectors and host strains, could be used. For example, Mav 203 yeast strain could be replaced by Clontech CG-1945 yeast strain and the pGAD and pGBD vectors could be replaced by the Clontech pGAD424 and pGBT9 vectors respectively.

Table 1: GAL4::Smad2 Interaction Verification

Co-Transformant	Strength
-	-
AD::Fast-1	+++
AD::Smad4	+++
AD::Smad1	-

MAV203 were transformed with GAL4::Smad2 and the indicated co-transformant. They were then assayed for strength of interaction on their ability to activate the URA3, HIS3, and LacZ reporter gene.

Table 2: BS69 Specificity Interaction

Co-Transformant	Strength
GAL4::Smad1	+/-
GAL4::Smad2	+++
GAL4::Smad3	+++
GAL4::Smad4	-
GAL4::Smad5	-

GAL4::Smad6	-
GAL4::Smad7	-
GAL4::PPAR-G	-

MAV203 were transformed with AD::BS69 and the indicated co-transformant and assayed for ability to interact. Strength of interaction is assayed by ability to activate URA3, HIS3, and LacZ reporter gene.

Example 2.

Confirming that BS69 interacts with Smad2 and Smad3 *in vivo*.

Co-immunoprecipitation and Western blot analysis.

The inventors have used pGen-Ires-neo as their chosen mammalian expression vector. The salient features of this construct are, in sequence: the backbone vector of plasmid pCI (Promega) with a CMV promoter for high level expression, a synthetic splice donor/acceptor sequence to ensure correct processing of transcripts, an engineered polylinker site to facilitate cloning of the gene to be expressed and the IRES element from encephalomyocarditis virus (gift from Ira Pastan; see Sugimoto et al., Biotechnology (1994) **12**:694) fused in frame to the initiating ATG of the neo gene from pcDNA3 (Invitrogen). It will be apparent to the person skilled in the art of mammalian expression that the use of this specific vector is not essential to the working of this example or the invention. Various alternative vectors, such as pIRES-neo (Clontech) and pCL-neo (Promega) could also be used.

PCR was used to create N-terminal Flag-tagged full length BS69 and truncated BS69 (amino acids 443 - 562), to be subcloned into pGen-Ires-neo expression vector. Full length hSmad-2, hSmad-3 without a tag, prepared according to Example 1, were also subcloned into pGen-Ires-neo.

The Flag octapeptide (5'-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-3'; IBI Flag Biosystem; SEQ ID No. 17) has a distinct anti Flag M1 monoclonal antibody binding site and has been carefully designed for easy access on the surface of the expression protein, allowing easy detection and purification.

PCR cloning strategy

		primer
Delta BS69	5'-EcoRI-NheI-ATG-aa.412	SEQ ID No. 18
Delta BS69 - Flag tagged	5'-EcoRI-NheI-ATG- Flag epitope-aa.412	SEQ ID No. 19
Full length BS69	5'-EcoRI-NheI-ATG-aa.2	SEQ ID No. 20
Full length BS69 - Flag tagged	5'-EcoRI-NheI-ATG- Flag epitope-aa.2	SEQ ID No. 21
BS69 3'end	3'-ClaI-XbaI-Stop codon	SEQ ID No. 22

BS69 untagged was cloned by RT-PCR from a human placenta cDNA library. Flag tagged BS69 was made by PCR from the full length untagged BS69. Truncated BS69 tagged and untagged constructs were made by PCR using the Yeast-two-hybrid cDNA clone identified above.

Cos-1 cells were transfected with respective DNAs using Lipofectamine Plus Reagent (Life Technologies). 48 hours after transfection, cells were treated with 15ng/ml of TGF-beta1 (R&D Systems) for 1 hour, and then lysed in 4 mls of ice cold RIPA buffer (150mls PBS, 1.5ml Triton-X-100, 0.75g sodium deoxycholate, 0.75ml 20% SDS). Cell lysates were collected by centrifugation and precleared with 1µg normal mouse IgG (Santa Cruz) and 20µl Protein A/G sepharose (Sigma). Co-immunoprecipitation was performed on 1 ml of lysate incubated at 4°C with 20µl of alpha-Smad2/3 polyclonal antibody (Santa Cruz) for 4 hours followed by the addition of 40µl Protein A/G Sepharose (Sigma). After overnight incubation at 4°C, the beads were spun down and washed 4 times with lysis buffer, then resuspended in 50µl Laemmli sample buffer (Sigma) and boiled for 5 minutes. Half of the sample was separated by polyacrylamide gel electrophoresis 4-15% Tris-HCl (Biorad) and transferred overnight to nitrocellulose membrane (Amersham). The filter was blocked in TBS buffer (20mM Tris base, 137mM NaCl, 3.8mM HCl, pH 7.6) with 0.1% Tween-20 and 5% non-fat powdered milk for 2 hours at room temperature, incubated with the primary antibody alpha-Flag monoclonal antibody (Santa Cruz) in a dilution of 1:2000 in TBS-Tween. The second antibody conjugated to HRP in a 1:1000 dilution for 1 hour. The filter was washed in TBS-Tween and detection was performed using enhanced chemiluminescence (Amersham).

It was found that both full length BS69 and truncated BS69 bind to hSmad-2, and to hSmad-3. This experiment therefore confirms that the BS69 interaction with hSmad-2/-3 identified in the yeast two-hybrid screening is a true *in vivo* interaction.

Example 3

Mammalian reporter assay.

pGen-Ires-neo vector was used for expression of untagged full length hSmad-2, hSmad-3, hSmad-4, BS69 and untagged truncated BS69.

The 959bp fragment corresponding to the promoter region (-811bp to +148bp) of the PAI-1 gene was PCRed from human genomic template DNA using SEQ ID Nos 23 and 24 primers. These primers allow addition of HindIII restriction sites at the 5'and the 3'ends to facilitate subcloning into the pGL3 basic vector (Promega - Luciferase reporter vectors - Technical manual, part#TM033).

The p300/CBP co-activator expression construct comprises the 1-7326bp nucleotide sequence of murine CREB Binding Protein, as published in Genbank (g435854), obtained by PCR from mouse brain mRNA using primers corrsponding to SEQ ID Nos. 25 and 26. The 7,326kb BamHI-NotI fragment was subcloned into pRc/RSV expression plasmid (Invitrogen).

On day one, 10^6 HepG2 cells (human hepatocellular carcinoma cells - Origin ECACC 85011430) were seeded per well of a 6 well plate in DMEM medium, 10%FCS, Glutamine and Penicillin/Streptavidin. Cells were transfected on day two by adding 4.75ug DNA/ well and 4ul of lipofectamine/ 4ul Reagent Plus (Life Technologies) per well as recommended by the manufacturer. Serum was added to the cells after 4 hours from. The medium was replaced with serum free medium the next day, and stimulated 7 hours later with 7.5ng/ml of TGF-beta1 overnight. Cytosol extract and dual luciferase assay were done the following day. The dual luciferase reporter assay system (Promega, technical manual part#TM040) was used as recommended by the manufacturer, and using 5ng of pBRL per well for normalisation of the assay.

The activity of the luciferase reporter gene relates to the activity of the PAI-1 promoter activation. PAI activity is minimal in the absence of TGF-β stimulation, but increases upon TGF-β stimulation. No effect of the above DNA constructs was observed in the absence of

TGF-β stimulation, therefore all the following experiments were done with TGF-β stimulation. When hSmad-2 or hSmad-3 were transfected alone into HepG2 cells, an additional increase in promoter activation was observed. There was no change when hSmad-4, p300/CBP, hSmad4+p300/CBP, fl BS69 or d-BS69 were transfected alone.

hSmad-4 is known to form a complex with activated Smad-2/or-3, and therefore additional hSmad-4 (limiting factor) together with hSmad-2/or-3 will increase PAI activity, as found in this assay and observed previously.

P300/CBPhas been shown to be a transciptional co-activator implicated in TGF-β signalling promoter activation, and when added to Smad-2/or-3 together with Smad-4 induces an additional increase in PAI activation (about 15%), as seen in our assay and observed previously.

Full length BS69 induces significant additional increase in PAI activation (up to 60%), when added to HepG2 cells together with hSmad-2/or-3, or hSmad-2/or-3 + hSmad-4 + p300/CBP.

Truncated BS69 acts like a dominant-negative form of BS69 when added to HepG2 cells together with hSmad-2/or-3, or hSmad-2/or-3 + hSmad-4 + p300/CBP. A small reduction of PAI activity is observed which probably correspond to the inhibitory effect of truncated BS69 to the full length constitutive basal expression of BS69 in HepG2 cells.

We have found using co-immunoprecipitation studies that both full length and truncated BS69 can bind Smad-2/or-3, and that full length BS69 is able to induce additional PAI promoter activation when tested in a mammalian reporter assay.

Claims

1. A method for identifying modulators of BS69 activity, which method comprises contacting an assay system, capable of presenting information on the effects of a test compound on the activity of BS69 or a derivative thereof, with a test compound and measuring the activity of BS69.

2. A method as claimed in claim 1, wherein BS69 activity refers to the ability of BS69 or a fragment thereof to bind to a BS69 binding protein.

3. A method as claimed in claim 2 wherein the BS69 binding protein is one selected from the group consisting of: Smad 2, Smad 3, a complex of Smad 2 and Smad 4, a complex of Smad 3 and Smad 4, or fragments thereof, or PAI-1 promoter element.

4. A cell or cell line comprising a reporter gene under the control of a BS69 transcription factor dependent promoter.

5. A method for identifying modulators of BS69 transcription which method comprises contacting a cell or cell line as claimed in claim 4 with a test compound, said cell or cell line supplied with exogenous or endogenous BS69, and determining the effect on BS69 transcription by the test compound by reference to enhanced or reduced expression of the reporter gene.

6. A method of treatment of a patient in need of such treatment for a condition which is mediated by the biological or pharmacological activity of BS69 on a human BS69 binding substrate, comprising administration of a polypeptide substantially as depicted in WO97/00323 SEQ ID NO:2 or a pharmacologically active fragment thereof.

7. A method of treatment of a patient in need of such treatment for a condition which is mediated by the biological activity of BS69 on a human BS69 binding substrate, comprising administration of a nucleic acid substantially as depicted in WO97/00323

SEQ ID NO:1 or the anti-sense sequence or a biologically-effective fragment of either thereof.

8. A compound that modulates BS69 transcription or other BS69 activity identified according to the method as described in any of claims 1, 2, 3 and 5.

9. A pharmaceutical composition comprising a compound that modulates the biological or pharmacological activity of BS69 on a human BS69 binding substrate identified according to the method as described in any of claims 1, 2, 3 and 5.

10. A method of treatment of a patient in need of such treatment for a condition which is mediated by the pharmacological or biological activity of BS69 on a human BS69 binding substrate comprising administration of a modulating compound or pharmaceutical composition as claimed in claims 8 or 9.

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single-stranded oligonucleotide primer sequence

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ggaaaagctt tctcctacct gaagttct 28

<210> 25
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
single-stranded oligonucleotide primer sequence

<400> 25
ggatccagaa tggccgagaa cttgctggac g 31

<210> 26
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
single-stranded oligonucleotide primer sequence

<400> 26
gcggccgcta caaaccctcc acaaactttt ctagtgtg 38

FOR UTILITY/DESIGN
CIP/IPCT NATIONAL/PLANT
ORIGINAL/SUBSTITUTE/SUPPLEMENTAL
DECLARATIONS

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PW
FORM
Z70421/UST

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the **INVENTION ENTITLED METHODS FOR IDENTIFYING MODULATORS OF BS69 ACTIVITY**

the specification of which (CHECK applicable BOX(ES))

X BOX(ES) → A ☐ is attached hereto

→ B ☐ was filed on _____ as U.S. Application No. _____

→ C ☐ was filed as PCT International Application No PCT/GB99 /03648 on 04 11 1999

and (if applicable to U.S. or PCT application) was amended on _____ I hereby state that I have reviewed and understand the contents of the above identified specification including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. Except as noted below, I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International Application which designated at least one other country than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International Application, filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed or (2) if no priority claimed, before the filing date of this application.

PRIOR FOREIGN APPLICATION(S) Number	Country	Day/MONTH/Year Filed	Date first laid-open or Published	Date Patented or Granted	Priority NOT Claimed
9824501.2	GB	10 11 1998			

Except as noted below, I hereby claim domestic priority benefit under 35 U.S.C. 119(e) or 120 and/or 365(c) of the indicated United States applications listed below and PCT international applications listed above or below and, if this is a continuation-in-part (CIP) application, insofar as the subject matter disclosed and claimed in this application is in addition to that disclosed in such prior applications, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of each such prior application and the national or PCT international filing date of this application:

PRIOR U.S. PROVISIONAL, NON PROVISIONAL AND/OR PCT APPLICATION(S) Application No. (series code/serial no.)	Day/MONTH/Year Filed	Status Pending, abandoned, patented	Priority NOT Claimed

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 16 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint Pillsbury Winthrop LLP, Intellectual Property Group, telephone number (202)861-3000 (to whom all communications are to be directed), and persons of that firm who are associated with USPTO Customer No.909 (see below label) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to delete from that Customer No. names of persons no longer with their firm, to add new persons of their Firm to that Customer No., and to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct the above Firm and/or an attorney of that Firm in writing to the contrary.

00909

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(include Zip Code)				

☐ OR ADDITIONAL INVENTORS see attached page.

☐ See additional foreign priorities on attached page (incorporated herein by reference).

Atty. Dkt. No. P _____
(M#)